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Investigation of the synovial fluid pro-inflammatory cytokines and clinical findings in disc displacements of TMJ

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ABSTRACT

Aims: Temporomandibular disorders (TMD) are common musculoskeletal disease that affects the soft tissues and bone structures of the temporomandibular joint (TMJ). Inflammatory mediators play a critical role in the etiology of TMD by affecting various molecular mechanisms. This study aims to investigate the effect of pro-inflammatory cytokines on TMJ internal derangements and evaluate the relationship between clinical features.

Methods: Patients who underwent arthrocentesis because of TMD were included in this study. Patients were divided into 2 groups according to anterior disc displacement with reduction (Group 1) and without reduction (Group 2). Clinical findings before and after the arthrocentesis were evaluated retrospectively. Synovial fluid TNF-α, IL-1β, and IL-6 levels were analyzed with the ELISA method.

Results: A total of 28 patients were included, with 15 patients in Group 1 and 13 patients in Group 2. All patients were female with an average age of 30.75±14.9. The average pre-operative pain score was 6.6±1.95 in all patients. After the arthrocentesis, the average pain score was 2.0 (1.0 - 2.5) and the difference was significant ($p<0.001$). Group 1 have a higher maximum mouth opening (MMO) than Group 2 preoperatively and the difference was statistically significant ($p=0.013$). The difference in synovial fluid cytokine levels was not statistically significant between groups. There was no statistically significant correlation between cytokines and clinical findings.

Conclusion: Arthrocentesis is beneficial for reducing joint pain in patients with TMJ internal derangements. Pro-inflammatory cytokine levels are similar in TMJs with disc displacement with and without reduction.

Keywords: Temporomandibular joint, TMJ, inflammatory, cytokine, synovial fluid

INTRODUCTION

Temporomandibular disorders (TMD) are a type of musculoskeletal disease that affects the soft tissues and bone structures of the temporomandibular joint (TMJ) and proceeds with functional abnormalities including muscle/joint discomfort and restricted jaw movement.[1] Internal derangements and osteoarthritis, characterized by the degenerations in cartilage and bone tissues, represents the most common forms of TMD. It has been observed that inflammatory mediators such as various cytokines or chemokines are released in the synovial fluid of patients with TMJ internal derangement or osteoarthritis, and there is also evidence of persistent inflammation and infiltration of inflammatory cells playing a critical role in the breakdown of articular structures within the synovium of osteoarthritis patients.[2,3] Hence, it becomes essential to assess the potential roles of cytokines and other mediators in illuminating the cellular and molecular events in the pathophysiology of TMJ internal derangement and osteoarthritis.

Treatment of TMD is based on conservative and surgical interventions. Patient education, dietary modifications, medical care, physical therapy, intraoral splint applications, and psychological therapy procedures are among the conservative treatment methods. Minimally invasive procedures are used when conservative treatment fails and clinical complaints continue. Intra-articular injections, intra-muscular injections, arthrocentesis, and arthroscopic lysis and lavage are minimally invasive techniques.[4] In the treatment of

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TMD, the physician should choose the least invasive and most effective treatment method for the patient, as repeated intra-articular interventions may reduce the chances of success. Therefore, arthrocentesis can be considered as the initial treatment option for patients who do not respond to conservative treatment. If arthrocentesis does not achieve success, arthroscopic surgery or other surgical interventions should be considered.[5] Synovial fluid samples taken during lavage can be used to evaluate TMJ disease and provide detailed information about inflammatory components in synovial fluid.[6] After the identification of arachidonic acid metabolites in painful TMJ, many other studies have been reported which provide an opportunity to evaluate composition changes to potentially diagnose and monitor response to treatment of TMJ disorders.[7,8] The first studies comparing cytokine levels in patients with internal derangement involving degenerative changes to healthy controls were published in the 1990s, and it was reported that patients had higher levels of TNF-α and IL-1β in synovial fluid compared to controls.[9] Another study reported elevated levels of IL-6 in 72% of patients with degenerative joint disease.[10] These studies demonstrate the role of TNF-α, IL-1β, and IL-6 in synovial inflammation. This study aims to investigate the effect of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) on TMJ internal derangements and evaluate the relationship between clinical features.

METHODS

The study was carried out with the permission of Erciyes University, Clinical Researches Ethics Committee (Date: 2019, Decision No: 556), and carried out at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry. All procedures were carried out by the ethical rules and the principles of the Declaration of Helsinki.

Patients who presented with TMJ complaints had a diagnosis, treatment, and follow-up were retrospectively reviewed for their suitability to participate in the study. Inclusion criteria for the study follow 1) Patients with complaints such as pain, tenderness, joint noises, and limited mouth opening in TMJ and clinically diagnosed with both anterior disc displacement with reduction (ADDwR) and anterior disc displacement without reduction (ADDwoR) according to the diagnostic criteria for temporomandibular disorders (DC/TMD) 2) Patients who received conservative treatment for TMD but did not show improvement in clinical findings, leading to the application of arthrocentesis 3) Patients from whom synovial fluid samples were obtained during arthrocentesis and with complete and well-documented medical records. Clinical diagnosis was also confirmed with magnetic resonance images (MRI). Patients with a history of trauma to the TMJ area and patients who underwent arthrocentesis and/or surgical interventions were excluded from the study. The medical records of patients who received treatment for TMJ internal problems were retrospectively reviewed between the years 2005 and 2019. Patients were divided into two groups according to the diagnosis. Group 1 consisted of patients with ADDwR and Group 2 consisted of patients with ADDwoR. For the study, the included patients' demographic data, dental history, diagnosis, clinical examination findings before and after arthrocentesis including maximum mouth openings (MMO), visual analog scale scores (VAS: 0-10), and follow-up information were gathered. Synovial fluid samples were used in the study from patients who underwent arthrocentesis procedures following the method described by Nitzan.11 During the arthrocentesis procedure, samples were collected by injecting and aspirating 2 ml of normal saline or Ringer's lactate solution into the upper joint space ten times. The levels of TNF-α, IL-1β, and IL-6 in synovial fluids were analyzed with ELISA (Enzyme-Linked Immuno Sorbent Assay) method. Findings were compared between groups.

Statistical Analysis

The statistical analysis of the data was performed using the Turcosa statistical software (Turcosa Analitik Ltd. Şti., www.turcosa.com.tr). The normal distribution of the data was evaluated using histograms, q-q plots, and the Shapiro-Wilk test. The homogeneity of variance was tested using the Levene test. For group comparisons, an independent two-sample t-test was used for parametric data, while the Mann-Whitney U test was used for non-parametric data. Within-group comparisons were conducted using a dependent two-sample t-test or Wilcoxon test. The data were expressed as mean ± standard deviation, median (1st and 3rd quartiles), or frequency (percentage). All analyses were performed with a statistical significance level of $p<0.05$, indicating statistical significance at a 95% confidence interval.

RESULTS

Preoperative Assessments

In the study, a total of 28 patients were included, with 15 patients in Group 1 and 13 patients in Group 2. All patients in the study were female with an average age of 30.75±14.9. The mean age was 30±16.4 in Group 1 and 31.6±13.5 in Group 2 ($p=0.781$). The average VAS score was 6.6±1.95 in all patients. Average MMO was measured 35.6± 5.8 mm in all patients before arthrocentesis.

Pre-operative VAS scores were found 6.8±1.4 in Group 1 and 6.3±2.4 in Group 2 and the difference was not significant (p=0.460). Maximum mouth openings (MMO) were compared and Group 1 have a higher MMO (38.86±5.98 mm) than Group 2 (33.53±4.31) and the difference was statistically significant (p=0.013).

Postoperative Outcomes

After the arthrocentesis procedure, the average VAS score was 2.0 (1.0 - 2.5) and MMO was 36.6±7.6 mm in all patients. The post-operative VAS score was statistically significantly lower (p<0.001). However, post-operative MMO was not statistically significant from preoperative values in all patients (p=0.316).

Post-operative VAS score was found 1.0 (1-2) in Group 1 and 2.0 (1-6) in Group 2 (p=0.164). Post-operative MMO was 38.3±8.06 in Group 1 and 35.5± 7.4 in Group 2 but the difference was not statistically significant (p=0.411). All data were shown in Table 1.

Table 1. Comparison of clinical findings between groups

	Pre-op VAS	Pre-op MMO(mm)	Post-op VAS	Post-op MMO(mm)
Group 1	6.8±1.4	38.86±5.98	1.0 (1-2)	38.3±8.06
Group 2	6.3±2.4	33.53±4.31	2.0 (1-6)	35.5± 7.4
P value	0.460*	0.013*	0.164†	0.411*

VAS: Visual Analog Scale, MMO: Maximum mouth opening, Data are given as median (1st-3rd quartiles) and mean ± standard deviation, *:Student t test, †: Mann Whitney U test

Synovial Fluid Analysis

Synovial fluid TNF-α, IL-1β, and IL-6 levels were compared according to the groups. The average levels were 148.75±22.5, 788.46±207.7 and 83.86±18.0 in Group 1 and 146.41±27.3, 735.53±172.6 and 90.0±16.9 in Group 2 respectively. The difference of synovial fluid cytokine levels were not statistically significant between groups (Table 2).

Table 2. Comparison of synovial fluid cytokines between groups

	TNF-α	IL-1β	IL-6
Group 1	148.75±22.5	788.46±207.7	83.86±18.0
Group 2	146.41±27.3	735.53±172.6	90.0±16.9
P value	0.806*	0.474*	0.365*

Data are given as median (1st-3rd quartiles), *:Student t test

The correlation between cytokines with preoperative MMO and VAS scores was investigated and there was no statistically significant correlation. A positive correlation was observed between TNF-α and IL-1β, as well as between TNF-α and IL-6. The relationship between TNF-α and IL-1β was positive, strong, and statistically significant (p<0.001, p=0.023). The correlation between IL-6 and IL-1β was not statistically significant (p=0.057) (Chart 1).

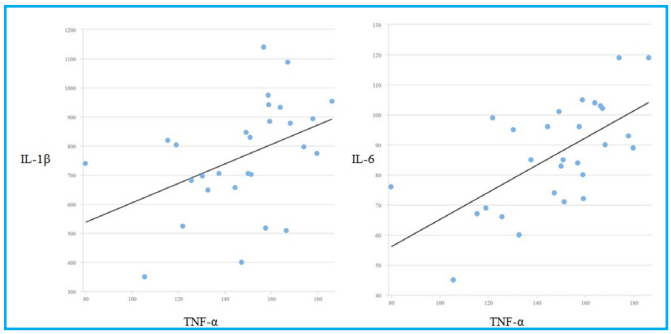

Chart 1. Correlations between IL-1β and IL-6 with TNF-α.

DISCUSSION

The goal of TMJ arthrocentesis is to use hydraulic pressure to wash away inflammatory mediators, release the articular disc, and dissolve adhesions between the disc surface and the articular fossa.[11] Arthrocentesis is a safe and rapid procedure used to treat various disorders affecting TMJ. Arthrocentesis is usually indicated when conservative and pharmacological methods have failed to provide satisfactory results.[6] With arthrocentesis, the intra-articular negative pressure is reduced, and adhesions are eliminated, allowing the disc to be detached from the glenoid fossa ceiling and facilitating condylar translation. Restoring healthy mandibular movements helps eliminate movement restrictions, thereby restoring the mouth opening to normal values.[5] Inflammatory cytokines and pain mediators are removed from the area through arthrocentesis, leading to the treatment of inflammation and alleviation of pain symptoms.[12] In this study, pain scores were measured with a Visual analog scale and it was observed that VAS scores were significantly decreased in all patients after arthrocentesis.

Although there are numerous studies on the pathophysiology of TMJ internal disorders, the exact mechanism of the disease remains not fully understood. In recent years, there are opinions suggesting that changes in the synovial fluid content caused by pro-inflammatory cytokines play a role in the etiology by affecting various molecular mechanisms.[13] In patients with TMJ internal derangement or osteoarthritis, increased hyperemia with capillary vessels and infiltration of inflammatory cells such as T cells or monocytes/macrophages can be visualized in the TMJ synovial membrane through arthroscopic or histopathological analysis.[13]

Cytokines are small proteins released by various cells in response to tissue damage and play a crucial role in cellular signaling. Among cytokines, there are interleukins, tumor necrosis factor, interferons, chemokines, and lymphokines. Some cytokines such as IL-1β, IL-6, IL-8, IL-12, and TNF-α are pro-inflammatory, while others like IL-4 and IL-10 are considered anti-inflammatory.[14]

Many of these cytokines have been found in the synovial fluids of patients with TMJ internal disorders and/or osteoarthritis, and they have been shown to control inflammation processes through various mechanisms. Therefore, these cytokines have been highlighted as promising therapeutic targets for TMJ diseases.[15-17]

Evaluation of the potential roles of cytokines and other mediators has become increasingly important and popular to elucidate the cellular and molecular events in the pathophysiology of TMJ internal disorders and osteoarthritis. In an experimental study conducted by Wang et al.[18] it was shown that in cases where the biomechanical properties of the disc are altered, the expression of IL-1β and TNF-α increased in TMJ, and this condition could impair the adaptive capacity of TMJ. In studies conducted with the biochemical analysis of TMJ synovial fluid, cytokines were investigated by analyzing synovial fluid obtained from patients with TME disorders and comparing it with the MRG (magnetic resonance imaging) images taken from the same patients.[19,20] It was shown that the levels of pro-inflammatory cytokines in synovial fluid were associated with the degree of joint effusion observed in the MRG images.[20] Similarly, it has been reported that the level of IL-6 in synovial fluid is associated with the severity of arthroscopic synovial inflammation.[21] These several investigations have shown that cytokines are involved in the inflammation of the synovium in TMJ. Therefore, in our investigation, based on the literature findings, it was deemed appropriate to use TNF-a, IL-1, and IL-6, which have previously been proven to be present in TMJ, to confirm the existence of inflammation in the retrospectively studied joints.

Synovial cytokines were compared in different patient groups in several studies. Ulmner et al.[22] reported that patients with disc displacement without reduction had higher cytokine concentrations including TNF-α and IL- 1β. On the other hand, Gulen et al.[23] investigated the synovial fluid cytokine levels in patients who had disc displacement with reduction and without reduction and it was reported that synovial fluid cytokine concentrations were not statistically significant between groups. In this study, we similarly observed that there were no statistically significant differences between groups. It is thought that the effect of tissue regeneration and remodeling processes in TMJ may lead to a shift in the dominance of pro-inflammatory cytokines in the early stages of the disease, and in later stages, a greater influence of anti-inflammatory cytokines might be observed. However, since anti-inflammatory cytokines were not investigated in our study, a direct comparison between pro-inflammatory and anti-inflammatory cytokines cannot be made.

The degree of inflammation in TMJ is crucial for the development of pain or clinical symptoms.[14] Some studies investigating IL-1β and TNF-α have shown a relationship between the synovial fluid content and the level of TMJ pain in patients with internal derangement and degenerative changes in TMJ.[8,9] It was reported that higher levels of TNF-α in the synovial fluid of patients with TMJ pain.[24] In another study, a correlation between joint effusion and IL-6 was demonstrated, and since joint effusion is associated with TMJ pain, it is suggested that IL-6 may be related to TMJ pain.[21] However, it is known that non-steroidal anti-inflammatory drugs (NSAIDs) reduce the levels of pro-inflammatory cytokines and the synthesis of prostaglandins. In a study, it was shown that COX (cyclooxygenase) inhibitors suppressed inflammatory mediators such as PGE2, IL-6, and IL-1β in TMJ synovial fluid.[25] Therefore, in studies related to cytokines in TMJ synovial fluid, it is recommended to discontinue NSAID use at least 7-14 days before the analysis.[23] In our study, we did not find any significant relation between clinical signs and cytokine concentrations. Since the study is retrospective, the records of the included patients regarding the use of non-steroidal anti-inflammatory drugs (NSAIDs) are not available. It is important to consider this limitation while interpreting the results and drawing conclusions from the study.

CONCLUSION

Arthrocentesis is beneficial for reducing joint pain in patients with TMJ internal derangements. Synovial fluid contains pro-inflammatory mediators which interact with each other's and pro-inflammatory cytokine levels in synovial fluid are not different in TMJs with disc displacement with and without reduction.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Clinical Researches Ethics Committee (Date: 2019, Decision No: 556).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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